Research Plan

On

INDUCED SYSTEMIC TOLERANCE IN BACTRIAL - TREATED CULTIVATED LEGUMINOUS PLANTS

For

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Introduction

India ranks second in worldwide in farm output out of which legumes rank third in world crop production which is mainly concerned to Indo-Gangetic Plains. Legumes are the genera and species of the Magnoliophyta family Fabaceae in the order Fabales. Legumes comprise lentils, peas, chickpeas, and beans. It finds major application as livestock forage and silage, grain, blooms, pharmaceutical/industrial, fallow/green manure and human consumption as these are the good source of protein and rich in iron and vitamin B complex. They also reduce carbon and nitrogen looses. Legumes are also cultivated in Rajasthan, a part of The Great Indian Desert known as the Thar Desert. Rajasthan the largest state of India constitutes 60% of its area in Thar Desert. Its vegetation includes floral diversity, with 911 wild species belonging to 780 genera and 154 families. Geographically, Rajasthan lies between 23° 3' to 30° 12' longitude and 69° 30' to 78° 17' latitude. It occupies 342,239 km^2 land area, which is 10.41% of the total land area of India. The Thar Desert lies between 24° to 28° N latitude and 68° to 71° E longitude, occupying an area of about 200,000 km^2. Enduring flora of the Thar Desert include tree and shrubs including cultivated leguminous plants, e.g., *Vigna* (*V. aconitifolia*, *V. mungo*, *V. radiata, V. unguiculata* etc.), *Pisum sativum*, *Cicer arietinum*, *Trigonella foenumgraecum, Cajanus cajan, Cymopsis tetragonoloba*, *Lens culinaris, Vicia faba, , Phaseolus lunatus, Lablab purpureus, Canovalia ensiforis, Arachis hypogea* etc. (http://dst.rajasthan.gov.in/). Legumes grown in arid and semi-arid regions of Rajasthan faces problem of abiotic stresses like salinity, alkalinity, high temperature and draught. Resulting in dehydration, osmotic stress etc. which caused a dramatic reduction in crop production globally.

The major limiting factors affecting the agricultural productivity worldwide are environmental stresses. Ecosystem of Rajasthan's arid and semi-arid regions is mainly affected by high temperature, soil salinity/alkalinity, low pH and metal toxicity. These stresses decrease the yield of crop and introduce barriers to the crop plants that are not suitable for the cultivation into that area (Hu, 2005). An understanding of controls over the below-ground function constitutes an important challenge as natural and agro-ecosystems (AESs) around the globe are exposed to anthropogenic pressures. In addition, the physico-chemical and structural properties of soils, including their development, have been greatly affected by the action of the rhizosphere (Choudhary et al., 2011). Plant growth-promoting bacteria (PGPB) that colonize the roots of

plants in the spermosphere (sphere that surround seed) enhance plant growth, after attaching to root surface. PGPB have been proved to be best eco-friendly remedy to accelerate the growth of plant in nutrient deficient soil with respect to chemical fertilizers which are least available to plant. PGPB solubilises nutrient and make them available to uptake by plants. There are some legumes like mung bean and soybean which are incapable of growing in draught and salt stresses as they may be devoid of mechanisms to survive in stressed conditions or unavailability of nutrient or increased secretion of ethylene hormone or decreased secretion of plant growth promoting hormones. This is the way how rhizospheric bacteria interact with plant tissues and cells with differing need (Barea et al. 2005). Apart from providing plant growth promoting activities PGPB also enables plants to survive in stress conditions by the mechanism known as Induced Systemic Tolerance (IST). IST is an important process to overcome the harmful effects of abiotic stress. It is a PGPB induced physical and chemical changes in plants that result in enhanced tolerance to abiotic stress viz; draught, salt, heavy metals, cold, heat shock and fertility stress. PGPB reduces the detrimental effects of stress by secreting stress releasing molecules. IST results from the production of plant growth promoting hormones like cytokinin, antioxidants like catalase, ACC deaminase activity or volatile organic compounds (Yang et al. 2009).

PGPB are a poorly investigated/unexplored group of microorganisms in arid and semi-arid zones of Rajasthan that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial arenas. The mechanisms through which PGPB exist and respond to their surroundings must be better understood in order to be more predictive about which higher plants to seek, study, and spend time isolating microfloral components. Exploitation of PGPB-plant interactions can result in the promotion of plant health and can play a significant role in low-input sustainable agriculture applications for both food and non-food crops.

Aim and Objectives of the study

This study mainly focuses on the induced systemic tolerance in bacterial-treated cultivated leguminous plants.

The objectives selected for this study are as follows:

1. Functional characterization of plant growth promoting properties of bacteria.
2. Develop bacterial mutant with enhanced plant growth promoting activities.
3. Molecular characterization of plant growth promoting activities of wild and mutant bacteria.
4. Molecular hybridization of stress responsive genes in bacteria and plant.
5. Role of bacteria in induced systemic tolerance for leguminous plants.

Review of literature

Plant growth promoting rhizobacteria (PGPR) are most widely studied group of PGPB that are now classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR) on the basis of their degree of association with plant root cells. The ePGPRs may exist in the rhizosphere or in the spaces between the cells of root cortex; iPGPRs locates generally inside the specialized nodular structure of root cells. In the last decade, bacteria belonging to different genera including *Azospirillum, Rhizobium, Pseudomonas, Pantoea, Burkholderia, Achromobacter, Bacillus, Microbacterium, Methylobacterium, Variovorax, Paenibacillus,*, and *Enterobacter* etc. have been reported to provide tolerance to different stress challenged host plants (Figure 1). Theses microorganisms can alleviate different stress releasing mechanisms and can open new dimension to stress challenged plants, thereby increasing the use of these microorganisms in agriculture to improve crop productivity (Martinez-Viveros et al 2010).

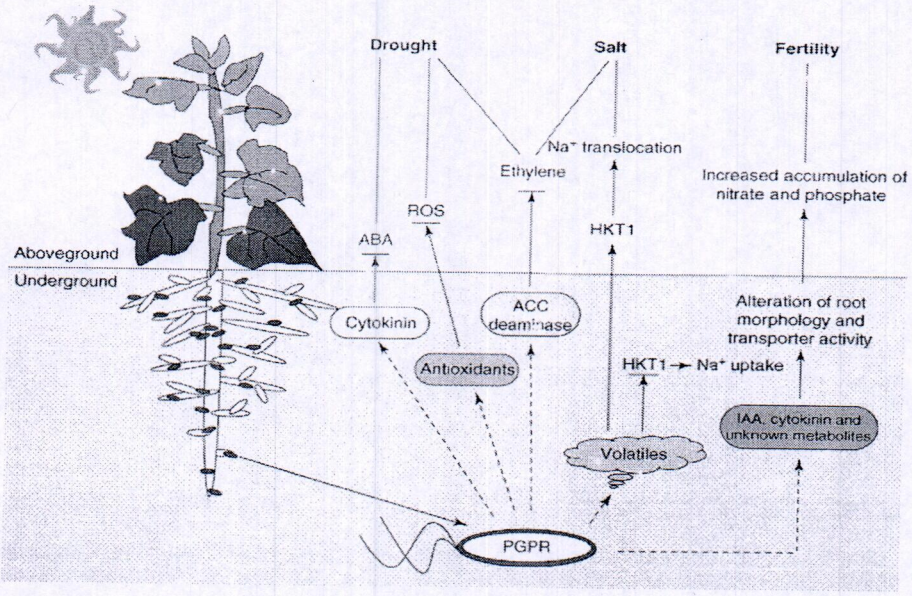

Fig. 1. PGPR operative mechanism under stress conditions. Yang et al., 2009, Trends in Plant Science, 14, 1-4

Production of indole acetic acid (IAA), and gibberellic acid (GA) results in increased root length, root surface area and number of root tips, leading to enhanced uptake of nutrients thereby improving plant health under stress conditions (Patten and Glick 2002; De-Bashan and Bashan; 2004; Mantelin and Touraine 2004; Adesemoye, A.O. et al. 2008; Egamberdieva and Kucharova

2009). Plant growth-promoting bacteria (PGPB) have been found to improve growth of tomato, pepper, canola, bean and lettuce under saline conditions (Yildirim and Taylor 2005; Barassi et al. 2006). Plant Stress Homeo-regulating Bacteria (PSHB) or PGPB were reported to be involved in secreting plant growth regulators, like cadaverine (Cassán et al. 2009), stress related phytohormone like abscisic acid (Cohen et al. 2008) and catabolism of stress hormone 1-aminocyclopropane-1 -carboxylate (ACC) by showing bacterial ACC deaminase activity in pepper (*Capsicum annuum* L.) and tomato (*Solanum Lycopersicum* L.) by *Achrompbacter piechaudii* ARV8 (Mayak et al. 2004). PGPB moreover make the plant to grow in nitrogen deficient soil by fixing nitrogen (Dobbelaere et al. 2003). These may also indirectly promote growth by production of antimicrobial compounds to decline or prevent the harmful effects of nonpathogenic, deleterious microorganisms (Raaijmaker et al. 2002) and siderophore production to scavenge iron and thereby reducing iron deficiency (Rajkumar et al, 2010).

During draught and salinity stress total water potential of plant cell changes which causes change in turgor pressure. This change is now maintained by accumulation of proline, glycine betaine, mannitol and sorbitol in transgenic plants that over produce such solutes to show tolerance to draught and salt stress (Chinnusamy et al. 2005). Salt tolerance in *Zea mays* coinoculated with *Rhizobium* and *Pseudomonas* was also experimented with increased production of proline along with decreased electrolyte leakage, maintenance of relative water content of leaves and selective uptake of K^+ ions (Bano and Fatima, 2009). Over expression of terhalose-6-phosphate synthase gene revealed upregulation of genes involved in stress tolerance and carbon and nitrogen metabolism. Thus, trehalose metabolism in rhizobia can be considered as a key for signaling plant growth, yield and adaptation to abiotic stress and its manipulation can have a major agronomical impact on leguminous plants (Suarez et al., 2008). Chen et al. (2007) correlated proline accumulation with drought and salt tolerance in plants. Introduction of *proBA* gene derived from *Bacillus subtilis* into *Arabidopsis thaliana* resulted in production of higher levels of free proline resulting in increased tolerance to osmotic stress in the transgenic plants. Grover et al. (2010) also described role of microorganisms in adaptation of agriculture crops to abiotic stress including mechanisms triggering osmotic responses and induction of novel genes in plants.

Figueiredo et al. (2008) reported coinoculation of *Rhizobium tropici* and *Paenibacillus polymyxa* increase plant growth, N content and nodulation of *Phaseolus vulgaris* L. under drought stress as

a result of over expressing trehalose-6-phosphate synthase gene. Co - inoculation of lettuce (*Lactuca sativa* L.) with *Pseudomonas mendocina* and arbuscular mycorrhizial fungi (*Glomus intraradices* or *G. mosseae*) alleviate the oxidative damage elicited by draught (Kohler et al. 2008).*Bacillus subtilis* GB03 strain was reported to emit volatile organic compounds (VOCs) that involved in IST (Ryu et al. 2004). Glutathione and ascorbate are involved in maintaining metabolic function of plants under water deficit conditions and thereby conferring protection. Low accumulation of these compounds in the lavender plants colonized by autochthonous drought tolerant *G. intraradices* indicated high drought tolerance in plants. Lavender plants inoculated with AM fungi showed improved water content, root biomas and N and K content (Marulanda et al. 2007).

The plant-associated habitat is a dynamic environment in which many factors may affect the structure and species composition of the bacterial communities that colonize plant tissues. Some of these factors are seasonal changes, plant tissue, plant species and cultivar, soil type and interaction with other beneficial or pathogenic microorganisms. An understanding of the structure and species composition of plant-associated bacterial populations is fundamental to understanding how plant-associated biological processes are influenced by environmental factors and, consequently, has important biotechnological implications. Plant growth and development cannot be adequately described without acknowledge microbial interactions.

Methodology

1. Maintenance and Glycerol stock preparation of bacterial strains

Recovered isolates will be maintained on yeast extract mannitol (YEM) agar plates amended with Congo red (Vincent, 1970) and plates will be incubated in an incubator at 28 ± 2°C for 24 to 36 hours. A 2.5 ml, autoclaved mixture of glycerol + YEM broth (1:1 v/v) was added to 5 ml of overnight grown culture in the same medium. One ml of this mixture will be suspended in cryovial and preserved at –20°C.

2. Functional Characterization

Bacteria will be functionally characterized for PGPB characteristic for IAA production (Gordon and Weber, 1951; Patten and Glick, 2002), ACC deaminase activity (Glick et al. (1999) with some modification, phosphate solubilization (Gaur, 1990) and siderophore production (Schwyn and Neilands, 1987).

3. Characterization of stress responsive molecules

Stress responsive molecules like proline, glycine beatine, ectoine etc will be checked in bacterium under stress condition (Bates et al., 1973).

4. Production of mutants

Bacterium will be treated with chemical mutagens like nitrosoguanidine according to Mishra and Goel (1999) and Mohamed and Ibrahim (2011) for high or no PGPR activities viz; phosphate solubilization (Gaur, 1990), siderophore production (Schwyn and Neilands, 1987) and IAA production (Glick et al. 1999).

5. Molecular characterization of plant growth promoting activities of wild and mutant bacteria

Promising strains will be selected and amplified using suitable primers. The amplified PCR product will then be sequenced for unique gene sequence and compared with the wild variety and the with sequences from the Gene Bank database of different bacterial strains, through the NCBI world Wide Website, at http://ncbi.nlm.bih-gov/BLAST.

6. Molecular hybridization of stress responsive genes

Stress responsive genes of the bacteria will be identified and molecularly hybridized to determine their alleviation in stress. mRNA will be extracted and separated by using gel electrophoresis. These separated bands will be now transferred to blotting membrane and treated with digoxigenin labeled probes.

7. Role of bacterium in IST

Selected strains will be analyzed *in vitro* for plant growth promoting activities in different leguminous plants that are normally challenged to stresses. PGPB inoculum will be prepared by harvesting bacterial cells from 24h grown cultures on YEM plates at 26°C. The inoculum will then be suspended in sterile physiological saline (0.9%) water to yield 10^8 colony forming units (cfu) per ml and inoculated in soil in controlled conditions and checked for their potency to promote plant growth under salt stress condition.

8. Publication of research results and thesis writing

Research results will be published in referred International and National journals, books and conferences.

Significance of work

- India ranks third in total legume production of world yet it is not sufficient for daily protein requirement of Indians so the used strategies the study may increase the yield of legumes and can help the country to achieve the needs of legumes.
- The study deals with the use of microbes to acclimatize the plants for stress induced physiological challenges. Hence, it is an eco friendly approach and declines the use of chemical fertilizers which causes biomagnifications and pollutes the ecosystem.
- Mutants having enhanced PGPR activities are developed which may help the plant to survive in stressed conditions.
- Molecular characterization of the gene may help in knowing the unique gene sequences that enhances the over producing characteristics for PGPR activities.
- The study will help in exploring the mechanisms involved in Induced Systemic Tolerance to plants.
- Selected bacterium can be used biofertilizer.

Future Prospects

- Soil nutrients are sometimes become unavailable to plants. Hence, the selected strains can be tested for field trials as a biofertilizer to make the soil nutrients available to plants.
- More study is required to know the mechanisms acquired by plants and microbes to tolerate the abiotic stresses of different geographical regions around the map.
- Signaling pathways should be determined to know how these molecules induce metabolic pathways to show IST.
- Protein is the main dietary supplement for human. Protein which we get from plant products is less in quantity as compared to animal protein. So, the attempts can also be made to increase the protein content of legumes using microbes.
- As transgenic approach is very much in trend so, mutated gene sequences can be used to produce transgenic plants and microbes showing higher PGPR activities.

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